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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Microwave assisted synthesis, antimalarial screening and structure–activity-relationship exploration of some phenylthiazolyl-triazine derivatives against dihydrofolate reductase

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Abstract In this study, a microwave-assisted methodology was first attempted to facilitate the synthesis of hybrid phenylthiazolyl-triazine derivatives. These two nuclei were clubbed together based on the structural requirement of existing antimalarial antifolates. A comparative analysis revealed that compounds synthesized using microwave-assisted procedure gave better yield and minimized the reaction time with respect to the conventional procedure. Hybrid compounds were screened for their in vitro anti-malarial activity against chloroquine-sensitive (3D-7) strain of *Plasmodium falciparum* at 5 µg/mL and 50 µg/mL dose level. An insight into the structure–activity-relationship of the synthesized compounds was gained by docking them in the crystal structure of wild type *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase.

Keywords Microwave · Thiazole · Triazine · Antimalarial · Docking

Introduction

Malaria is the fifth cause of death from infectious diseases worldwide after respiratory infections, HIV/AIDS, diarrheal diseases and tuberculosis and the second in Africa, after HIV/AIDS. Despite of the urgent need for a new anti-malarial agent, drug discovery for malaria is still very challenging mainly because of the increasing resistance arising to existing antimalarial drugs like sulphadoxine, pyrimethamine, artemisinin, etc(World Health Organization, 2015). Although with continuing evolution of the drug discovery methods, high quality lead generation processes may deliver compounds which are more likely to succeed in later stages (Ratti and Trist, 2001).

Thiazoles are important biologically active compounds because of their wide range of activity. They are used as anti-inflammatory (Bekhit et al., 2010), anti-bacterial (Maruyama et al., 2007), anti-microbial (Morales-Bonilla et al., 2006), anti-tumor (Choi et al., 2011), anti-tubercular (Prasanna et al., 2010), diuretic (Andreani et al., 1987), insecticidal (Gupta et al., 2010), muscle relaxant (Pandey et al., 2004), local anesthetic (Badiger et al., 2012), anti-malarial (Gahtori et al., 2012) agents etc. On the other hand 1,3,5-triazine derivatives are used for synthesizing various medicinally important antimalarial compounds (Rastelli et al., 2000). Both these nuclei were hybridized based on the structural requirement of existing antimalarial-antifolates (Legesse and Prasad, 2011), which was well satisfied by the phenyl ring as the hydrophobic tail, thiazole nuclei as the linker and triazine as the hydrogen bond donor head group. Dihydrofolate reductase enzyme of *Plasmodium falciparum* was targeted for this study as this is a key enzyme in the reduction of dihydrofolate to tetrahydrofolate, which is essential for parasite's DNA synthesis (Nzila, 2006; Anderson and Wright, 2005). The structural modifications of 1,3,5-triazine posed a great challenge for synthesis. The chlorine atoms of 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) can be substituted with different amines at different temperatures. In general, first chlorine can be

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substituted at 0–5 °C, second one at 40–45 °C and third one at 100–120 °C (Afonso et al., 2006). The stepwise substitution of the chlorine atoms in cyanuric chloride occurs smoothly only for the first two chlorine atoms in aqueous dioxane at 40–45 °C. However, the substitution of the third chlorine atom at higher temperature under similar conditions may lead to hydrolysis. Therefore, substitution of the third chlorine atom could be carried out in pure dioxane (Baindur et al., 2005; Kuo et al., 2005). The conventional synthesis of hybrid phenyl pyrazzole-triazine derivatives (Katiyar et al., 2005), aminoquinoline-triazine hybrids (Kumar et al., 2011; Sahu et al., 2016), and phenyl thiazole-triazine derivatives (Gahtori et al., 2012) are well documented. The syntheses of these hybrids were achieved by combining two nuclei at the reflux temperature of dioxane and the substitution of the third chlorine atom requires almost 6–8 hours.

Microwave-assisted organic synthesis is an invaluable technology for medicinal chemistry and drug discovery since it greatly reduces reaction time, typically from days or hours to minutes or even seconds (Kappe and Stadler, 2006; Ma et al., 2010).

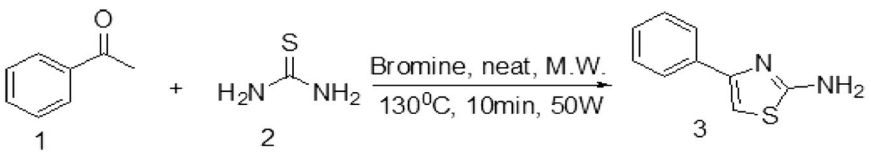
To facilitate the synthesis of hybrid phenyl thiazolyl-triazine derivatives and also to minimize the impurities owing to high temperature, long-term open vessel reactions, here in the present work, we report some successful microwave-assisted replacement of second and third chlorine atoms of this class of compounds. To reveal their possible antimalarial activity, synthesized compounds were screened against 3D7 strain of *Plasmodium falciparum*. The synthesized compounds were also docked into the crystal structure of wild-type *Plasmodium falciparum* Dihydrofolate Reductase Thymidylate Synthase (*Pf*-DHFR-TS) to get an insight of their structure–activity-relationship (SAR).

Materials and methods

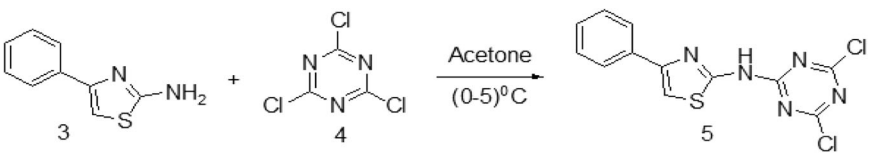
Chemistry

Compound 3 and compound 5 were synthesized in accordance with the reported procedures (Schemes 1 and 2) (Afonso et al., 2006; Cáceres-Castillo et al., 2012). Compound 5 was allowed to react with propylamine under pressure in 10 ml closed vessels at i) 100 °C temperature, 80 Watt power and ii) 120 °C temperature, 100 Watt power in CEM Microwave Synthesizer since higher temperature is required to replace the second and third chlorine atoms of triazine molecule (Legesse and Prasad, 2011).

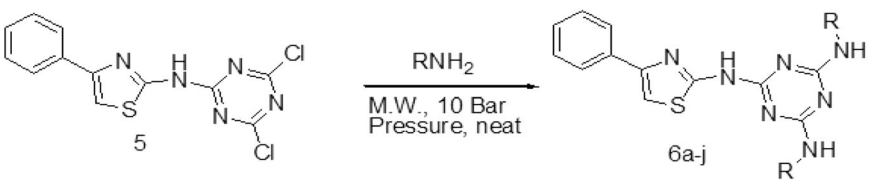
Further in order to optimize the reaction condition and also to determine the maximum yield, the reactions were carried out for 3, 5, 7, 9, and 11 min in both the cases (Scheme 3). Since the reactions were carried out under neat condition, amines were taken in excess (for 0.1 g of compound 5, 2 ml of amine was taken). Maximum yield was obtained at 120 °C, 100 W, 10 bar pressure and 9 min of microwave irradiation (Table 1). After the reaction, whole content of the reaction vessel was poured into water and filtered. The residue thus obtained was washed several times with water to remove the excess amine, dried in hot air oven at 50–60 °C and purity was established by recrystallizing the products in chloroform, separation of impurities using



Scheme 1 Synthesis of 2-amino-4-phenylthiazole from acetophenone, thiourea, and bromine(Cáceres-Castillo et al., 2012)



Scheme 2 First chlorine substitution of cyanuric chloride by 2-amino-4-phenylthiazole in conventional way



Scheme 3 Second and third chlorine substitution by propylamine in Microwave under pressure

Table 1 Optimization of reaction conditions with propylamine

Entry	Temperature (°C)	Power (W)	Time (min)	% yield
1	100	80	3	28.89
2	100	80	5	29.56
3	100	80	7	32.87
4	100	80	9	35.63
5	100	80	11	30.44
6	120	100	3	38.77
7	120	100	5	40.53
8	120	100	7	62.46
9	120	100	9	65.09
10	120	100	11	59.83

Table 2 Second and third chlorine substitution by different amines in microwave at 120 °C, 100 W, 9 min, and 10 bar pressure

Entry	Product	RH_2	Conventional yield[a] (%)	Microwave yield (%)
1	6a	Cyclopropylamine	30.11	52.12
2	6b	Propylamine	34.63	65.09
3	6c	Dimethylamine	25.33	57.81
4	6d	Methylamine	26.92	50.55
5	6e	Aniline	24.23	45.50
6	6f	Piperidine	Nil	28.82
7	6g	Cyclohexylamine	Nil	28.27
8	6h	p-Toluidine	Nil	32.64
9	6i	o-Toluidine	30.87	67.64
10	6j	Benzylamine	31.24	65.01

[a] Yield after 8 h of conventional reflux in dioxane

column chromatography, comparing the R_f values by thin layer chromatography and melting point determination by open capillary method. The structures of the synthesized compounds were ascertained on the basis of their Fourier transform infrared (FTIR), ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and mass spectroscopic analysis.

With this result, we extended our investigation using various amines (Table 2) under 120 °C, 100 W, 10 bar pressure, and 9 min of microwave irradiation. It had already been revealed by our previous work that –NH linkers between triazine nuclei and terminal substituents have more affinity towards the binding site than mercapto analog (Gahtori et al., 2012), so different aliphatic, aromatic, cyclic, and aryl amines were undertaken for this study. For comparing the yields in microwave and in conventional syntheses, the reactions were carried out in dioxane under reflux, with same amount of amine but for a longer duration of 8 h. Based on their melting point, R_f value and FTIR data the products obtained from conventional and microwave synthesis were confirmed to be the same.

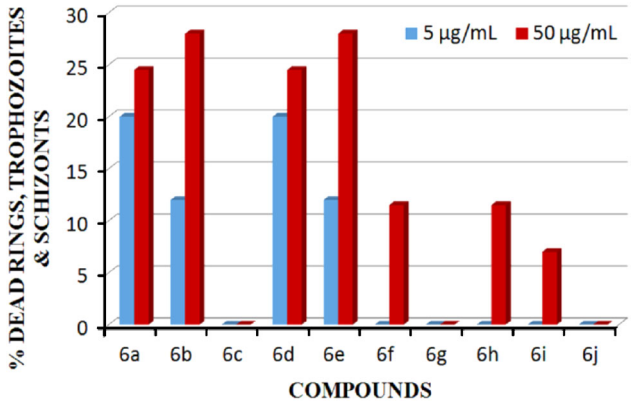


Fig. 1 Graphical representation of the in-vitro antimalarial activity data

Antimalarial screening

Chloroquine sensitive 3D7 strain of *P. falciparum* were maintained routinely in stock cultures in medium RPMI-1640 supplemented with 25 mmol 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1 % D-glucose, 0.23 % sodium bicarbonate, and 10 % heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5 % D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, the initial ring stage parasitaemia of 0.8–1.5 % at 3 % hematocrit in a total volume of 200 µL of medium RPMI-1640 was uniformly maintained.

The in vitro antimalarial assay was carried out according to microassay of Rieckmann et al. (1978) in 96 well-microtitre plates, with minor modifications. A stock solution of 5 mg/mL of each of the test samples was prepared in dimethyl sulfoxide (DMSO) and subsequently diluted with the culture medium. The test compounds in 20 µL volume concentration at 5 µg/mL and 50 µg/mL in a duplicate well were incubated with parasitized cell preparation at 37 °C in a candle jar. After 36–40 h of incubation, the blood smears were prepared from each well and stained with Giemsa stain. The level of parasitemia in terms of % dead rings along with trophozoites and schizonts was determined by counting a total of 100 asexual parasites (both live and alive) microscopically using chloroquine as the reference drug (Fig. 1). With each run, reference compound viz. chloroquine (0.7 µg/mL) was maintained at IC_{50} dose and the results were recorded as mean of two replicates.

SAR exploration

The crystal structure of wild type *Pf*-DHFR-TS complex was obtained from protein data bank (1j3i). 1j3i was selected for this study as 93.9 % (1025/1092) of all residues of this protein were in favored (98 %) regions and 99.4 %

Table 3 Heavy Atom RMSD to WR99210 X-ray pose

Entry	Pose[a]	RMSD (Å)
1	1	0.9770
2	2	0.9711
3	3	1.5187
4	4	1.3163
5	5	1.1196
6	WR99210 X-ray pose	0.0000

[a] 1, 2, 3, 4, and 5 were the five docked poses of WR99210 by the protocol used in the study

Table 4 Binding energy and dock scores of the products, WR99210 and Choroquine

Product	Binding energy (kCal/mol)	Dock Score
6a	−9.72	73.19
6b	−22.18	68.46
6c	−28.07	73.48
6d	−4.63	57.64
6e	−34.58	77.70
6f	−123.44	76.06
6g	−19.39	77.92
6h	−43.68	73.36
6i	−34.2	75.49
6j	−65.53	79.82
WR99210	−97.21	20.76
Chloroquine	−72.93	51.47

(1085/1092) of all residues were in allowed (>99.8 %) regions of Ramachandran plot with satisfactory resolution of 2.33 Å (Lovell et al., 2003). In the protein workspace of Accelrys Discovery Studio Version 2.5, water molecules, co-crystallized ligand WR99210 were removed and cofactors nicotinamide adenine dinucleotide phosphate, deoxyuridine monophosphate were retained. Since the prototype WR99210 was bound to chain A, so only chain A of the protein was used in the present work. This refined protein was simulated in the workspace by applying CHARMm forcefield. Docking was done using CDOCKER of Accelrys Discovery Studio version 2.5. The center of co-crystallized ligand WR99210 was selected as the binding site and a sphere (28.0015, 5.89121, 59.8342, 16.1) was created around it to define the active site. The protocol was validated by calculating root mean square deviation (RMSD) between the docked pose of WR99210 and ligand's X-ray docking pose, which was found to be in between 0.9711 and 1.5187 Å (Guosheng et al., 2003) (Table 3). Binding energies and dock scores of the final compounds were given in Table 4 and their docked poses in the active site of *Pf*-DHFR-TS were given in Fig. 2.

Result and discussion

Chemistry

It was found that the microwave-assisted process offers higher yield as compared to the conventional one (Table 2). It depicts that the percentage yield of seven compounds synthesized by the conventional procedure remains within 24–35 %. All these compounds when synthesized by the microwave-assisted process, the percentage yield had exceeded up to 67 %. Remaining three compounds which could not be synthesized by the conventional procedure had 28–32 % yield in the microwave-assisted procedure. In addition, the microwave-assisted reactions were of very short duration as compared to conventional synthesis for all the amines undertaken in this study. Shorter reaction time minimizes the possibilities of thermal decomposition resulting in higher yield of the final products.

Antimalarial screening

The antimalarial activities of the synthesized compounds were determined in terms of % dead rings, trophozoites, and schizonts. Out of the ten compounds, seven were mild to moderately active against 3D7 strain of *P. falciparum* (Fig. 1). Out of a total ten compounds, only 6a, 6b, 6d, and 6e were active at lower 5 µg/mL dose level. Only seven compounds were active at higher 50 µg/mL dose. Three compounds 6c, 6g, and 6j had not shown any activity even at 50 µg/mL dose levels.

SAR exploration

The extent of docking can be understood from the binding energy and dock score values given in Table 4. Binding energies of the synthesized compounds were within the range of −123.44 to −4.63. Dock scores of the synthesized compounds were within the range of 57.64 to 79.82, which were sufficiently higher than that of the reference compounds WR99210 and chloroquine. From the in vitro antimalarial activity data (Fig. 1) and the molecular docking study (Fig. 2) it was observed that the compounds, which were active at 5 µg/mL dose level showed pi-cationic and pi-sigma interaction with Arg 122 and Ile112 in both the compounds 6a and 6b respectively; H-bonded interaction between Asp 54, –NH of triazine ring and –NH of methylamine of compound 6d; pi-pi interaction between Phe 116 and one of the aniline rings of compound 6e present as a substitution in its triazine ring. Other three out of the seven active compounds, 6f, 6h, and 6i showed the presence of pi-cationic interaction between Phe 58 and positively charged *N*-atom of triazine ring; pi-pi interaction between Phe 58, Phe 116, and p-toluidine; pi-pi interaction

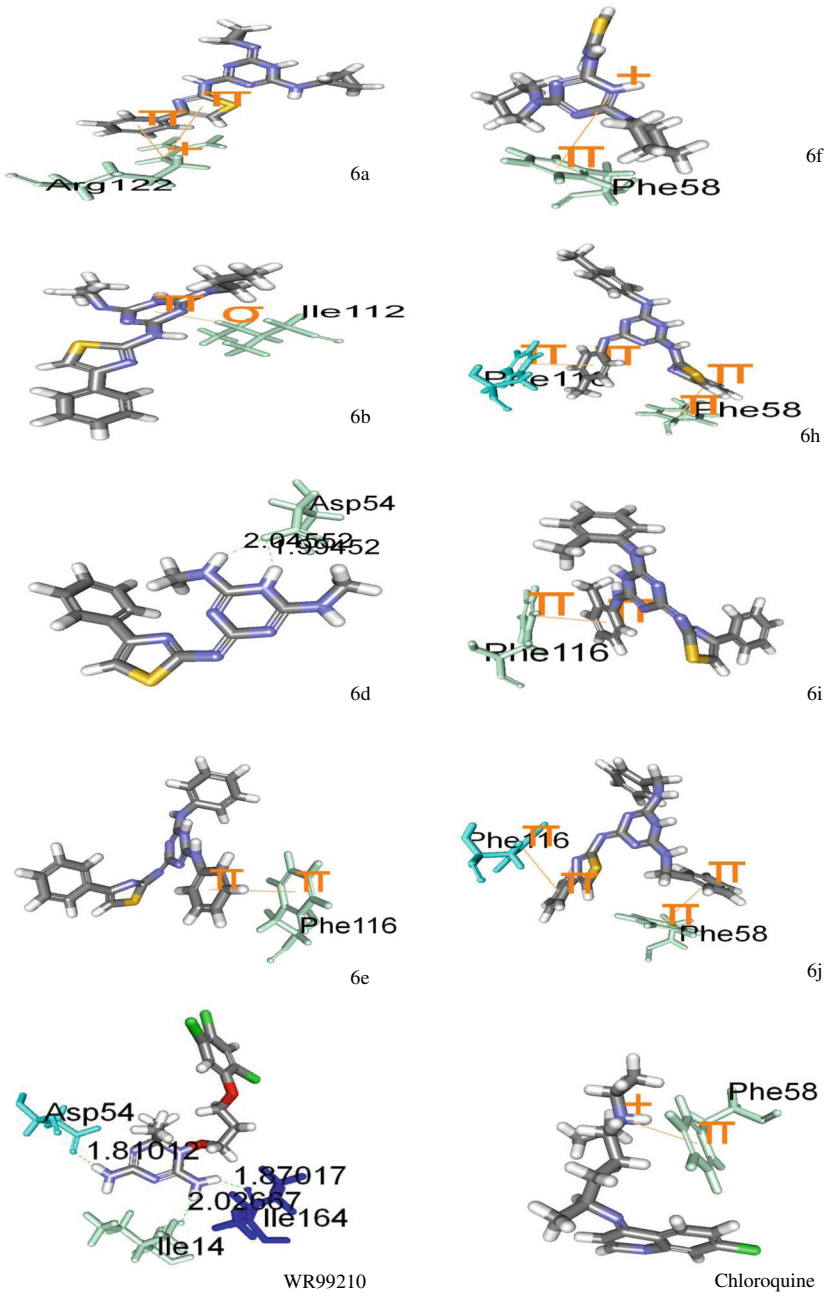


Fig. 2 Docking poses of the synthesized compounds, WR99210 and chloroquine in the active site of *Pf*-DHFR-TS

between Phe 116 and o-toluidine present as a substitution in the triazine ring respectively. The phenyl ring of phenylthiazole part, in compound 6h and 6j had also participated in formation of pi-pi interaction with Phe 58 and Phe 116, respectively. Two among the three totally inactive compounds 6c and 6g had not shown any kind of interaction in the active site of the target protein. Thus, the SAR of the synthesized compounds reveal that in most of the active compounds, the triazine ring or the different amino substitutions present in it had well played a significant role of hydrogen bond donor head group. Whereas the pi-pi interaction formed by the phenyl ring of the phenylthiazole moiety was evident of their hydrophobic behavior and the absence of any sort of interaction with thiazole ring, well justifies the selection of this ring as a linker between the other two moieties.

Cyclopropylamine and methylamine substituted hybrids (6a & 6d) had shown same amount of percent dead rings but only methylamine was involved in H-bonding. The bulkiness of other primary amines might inhibit them in forming such interactions. Aromatic primary amines like aniline, o-toluidine, p-toluidine were involved in pi-pi interaction. Among them aniline containing hybrid (6e) had good antimalarial activity.

It has also been observed that the electron donating groups with secondary nitrogen viz. Cyclopropyl (6a), propyl (6b), methyl (6d), and aromatic amines (6e) enhanced antimalarial activity, whereas tertiary amine substituted compounds showed no antimalarial activity. Further in case of aromatic amines there should not be any other substitution (6h, 6i) on the ring.

All the synthesized compounds were found to have a higher dock score than that of WR99210 and chloroquine. But the docked pose of chloroquine had shown only the presence of weak pi-cationic interaction with Phe 58. It may be due to noninvolvement of chloroquine with *Pf*-DHFR-TS in its mechanism of action making its in-silico in-vitro correlation insignificant.

Moreover if the binding pattern of the synthesized compounds were compared with that of WR99210 (Fig. 2), it can be seen that Asp 54 was the only common residue between WR99210 and 6d. Compounds 12b, 29d and 21c of same hybrid synthesized previously by our group (Gahtori et al., 2012) had also shown H-bonded interaction with Asp 54 and Ile 164, which supports this claim. The best compound 34d reported by Gahtori et al. (2012) had shown interaction with Arg 122. Presence of such residues in the binding site of the synthesized compounds 6a and 6d can also be the reason for their good antimalarial activity. Enhancement of antimalarial activity in the compounds 6a, 6b, 6d, and 6e well justifies the selection of amines over mercapto analog present in the previously reported lead compound 34d.

Spectroscopic data

*N^2, N^4-dicyclopropyl-N^6-(4-phenylthiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**6a**)*

Color: cream; M.p: 124–127 °C; R_f. 0.45 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 262 nm; FTIR (cm^{-1}): 3432.87, 3235.42 (N–H stretch), 3113.05, 3014.26 (C–H aromatic stretch), 1593.56 (N–H bend), 1482.39, 1440.94 (C–C aromatic stretch), 1329.50, 1306.57, 1279.96 (C–N aromatic stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4043, 7.3283, 7.2617 (CH, benzene, $J = 1.11$ Hz), 7.0153 (CH, thiazole, $J = 0.43$ Hz), 4.1484 (NH, aromatic, $J = 0.77$ Hz), 1.2541 (CH, cyclopropyl, $J = 1.05$ Hz), 0.6607, 0.5187 (CH_2, cyclopropyl, $J = 2.82$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.89, 134.8, 128.47, 127.77, 126.68, 24.07, 23.59, 7.66, 6.99, Mass: 366.12 $(M+H)^+$.

*N^2-(4-phenylthiazol-2-yl)-N^4,N^6-dipropyl-1,3,5-triazine-2,4,6-triamine (**6b**)*

Color: white; M.p.: 163–167 °C; R_f. 0.55 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 265 nm; FTIR (cm^{-1}): 3261.07 (N–H stretch), 2959.66, 2929.77 (C–H aromatic stretch), 1631.70 (N–H bend), 1470.53, 1442.89 (C–C aromatic stretch), 1326.27, 1294.63, 1271.99 (C–N aromatic stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4159, 7.3785, 7.2590 (CH, benzene, $J = 2.31$ Hz), 6.9908 (CH, thiazole, $J = 1.11$ Hz), 3.5699 (NH, aromatic, J = 1.62 Hz), 1.6941 (CH_2 methylene, $J = 3.80$ Hz), 1.0118 (CH_3 methyl, J = 3.02 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.64, 162.19, 161.43, 149.82, 135.24, 128.55, 127.78, 126.55, 107.78, 43.31, 23.21, 11.57; Mass: 370.23 $(M+H)^+$.

*N^2, N^2, N^4, N^4-tetramethyl-N^6-(4-phenylthiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**6c**)*

Color: white; M.p.: 204–207 °C; R_f. 0.54 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 262 nm; FTIR (cm^{-1}): 3237.07 (N–H stretch), 3098.98, 2922.58, 2859.34 (C–H aromatic stretch), 1586.25 (N–H bend), 1454.34, 1442.57 (C–C aromatic stretch), 1299.96, 1275.95 (C–N aromatic stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4038, 7.3853, 7.2603 (CH, benzene, $J = 2.28$ Hz), 7.0331 (CH, thiazole, $J = 1.13$ Hz), 3.1703 (NH, aromatic), 2.1710 (CH_3 methyl, $J = 3.22$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.27, 161.67, 159.33, 134,86, 128.57, 127.61, 125.99, 106.87, 36.18; Mass: 342.12 $(M+H)^+$.

*N^2, N^4-dimethyl-N^6-(4-phenylthiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**6d**)*

Color: yellow; M.p: 115–119 °C; R_f. 0.47 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 270 nm; FTIR (cm^{-1}): 3265.41 (N–H stretch), 3011.43, 2931.03 (C–H aromatic stretch), 1597.54 (N–H stretch), 1467.66, 1438.44 (C–C aromatic stretch), 1333.74, 1276.57 (C–N aromatic stretch); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 7.4684, 7.3450, 7.3255, 7.2454 (CH, benzene, $J = 5.42$ Hz), 6.8560 (CH, thiazole, $J = 0.53$ Hz), 3.7137 (NH, aromatic, $J = 2.54$ Hz), 2.5773 (CH_3 methyl, $J = 2.32$ Hz); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 168.38, 148.72, 134.12, 133.93, 128.20, 127.14, 125.41, 101.11, 26.99; Mass: 314.19 $(M+H)^+$.

*N^2, N^4-diphenyl-N^6-(4-phenylthiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**6e**)*

Color: white; M.p: 253–256 °C; R_f. 0.55 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 210 nm; FTIR (cm^{-1}): 3404.30 (N–H stretch), 3023.18, 2871.90 (C–H aromatic stretch), 1593.02 (N–H bend), 1466.57, 1432.12 (C–C aromatic stretch), 1324.61, 1297.53, 1203.23 (C–N aromatic stretch); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 7.3903, 7.3756, 7.3101, 7.0209 (CH, benzene, $J = 4.95$ Hz), 6.9160 (CH, thiazole), 3.3748, 3.4250 (NH, aromatic); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 163.76, 162.13, 159.29, 149.11,

134.58, 128.08, 127.23, 125.54, 107.56, 99.49; Mass: 438.24 $(M+H)^+$.

N-*(4,6-di(piperidin-1-yl)-1,3,5-triazin-2-yl)- 4-phenylthiazol-2-amine (**6f**)*

Color: white; M.p: 207–209 °C; R_f. 0.40 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 262 nm; FTIR (cm^{-1}): 3224.24 (N–H stretch), 2925.71, 2848.23 (C–H aromatic stretch), 1599.50 (N–H bend), 1491.69, 1436.92 (C–C aromatic stretch), 1347.95, 1299.34, 1237.15 (C–N aromatic stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.4114, 7.3735, 7.2609 (CH, benzene, $J = 2.14$ Hz), 7.0344 (CH, thiazole, $J = 1.07$ Hz), 3.7856 (NH, aromatic, J = 9.07 Hz), 1.6068 (CH_2, piperidinyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.58, 162.07, 128.59, 127.63, 125.99, 106.79, 25.81, 24.91; Mass: 422.22 $(M+H)^+$.

N^2, N^4*-dicyclohexyl-*N^6*-(4-phenylthiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**6g**)*

Color: white; M.p: 186–188 °C; R_f. 0.57 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 262 nm; FTIR (cm^{-1}): 3269.66 (N–H stretch), 2927.48, 2852.38 (C–H aromatic stretch), 1623.76 (N–H bend), 1497.24, 1468.63 (C–C aromatic stretch), 1338.83, 1302.46, 1275.39 (C–N aromatic stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.3682, 7.2611, 7.1033 (CH, benzene, $J = 2.22$ Hz), 6.9971 (CH, thiazole, $J = 1.00$ Hz), 4.0669 (NH, aromatic, $J = 0.91$ Hz), 2.1725 (CH, cyclohexyl, $J = 2.76$ Hz), 1.7869, 1.6485, 1.4860, 1.2543 (CH_2, cyclohexyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 128.58, 127.71, 126.63, 33.30, 25.70, 25.14; Mass: 450.22 $(M+H)^+$.

N^2*-(4-phenylthiazol-2-yl)-*N^4,N^6*-di p-tolyl-1,3,5-triazine-2,4,6-triamine (**6h**)*

Color: cream; M.p.: 260–264 °C; R_f. 0.53 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 270 nm; FTIR (cm^{-1}): 3394.81 (N–H stretch), 3022.72, 2914.69, 2856.64 (C–H aromatic stretch), 1627.91, 1599.53 (N–H bend), 1498.72, 1462.65, 1408.21 (C–C aromatic stretch), 1356.15, 1289.10 (C–N aromatic stretch); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 7.4003, 7.2858, 7.1040, 6.8222 (CH, benzene), 6.4966 (CH, thiazole) 4.0669 (NH, aromatic, $J = 0.39$ Hz), 2.5273, 2.3098 (CH_3 methyl, $J = 6.62$ Hz); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 149.03, 134.62, 129.07, 128.58, 128.35, 125.55, 114.12, 20.45; Mass: 466.21 $(M+H)^+$.

N^2*-(4-phenylthiazol-2-yl)-*N^4,N^6*-di o-tolyl-1,3,5-triazine-2,4,6-triamine (**6i**)*

Color: cream; M.p.: 249–252 °C; R_f. 0.53 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 270 nm; FTIR (cm^{-1}): 3382.87, 3261.19 (N–H stretch), 3051.57, 3026.26, 2941.05 (C–H aromatic stretch), 1620.76 (N–H bend), 1500.50, 1450.09, 1414.28 (C–C aromatic stretch), 1285.74 (C–N aromatic stretch); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 7.4292, 7.3010, 7.1820, 6.8930, 6.6164 (CH, benzene), 6.4774 (CH, thiazole, $J = 0.41$ Hz), 4.6893 (NH, aromatic, $J = 0.64$ Hz), 2.2537 (CH_3 methyl, $J = 6.37$ Hz); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 164.92, 162.20, 159.66, 148.84, 145.94, 136.92, 129.98, 128.17, 127.09, 126.82, 113.98, 18.06, 17.26; Mass: 466.27 $(M+H)^+$.

N^2, N^4*-dibenzyl-*N^6*-(4-phenylthiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (**6j**)*

Color: white; M.p.: 217–220 °C; R_f. 0.42 (Hexane: $CHCl_3$:: 4:1); UV λ_{max} ($CHCl_3$): 262 nm; FTIR (cm^{-1}): 3422.99 (N–H stretch), 3023.28, 2921.90 (C–H aromatic stretch), 1632.81, 1598.38 (N–H bend), 1451.10 (C–C aromatic stretch), 1297.56 (C–N aromatic stretch); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 7.3885, 7.3698, 7.2810, 7.1958 (CH, benzene), 6.4102 (CH, thiazole), 4.6735 (NH, aromatic, $J = 1.36$ Hz), 1.7295 (CH_2, methylene); ^{13}C NMR (100 MHz, DMSO) δ (ppm): 166.43, 165.61, 148.87, 140.02, 134.58, 128.28, 127.94, 126.81, 99.49, 40.18; Mass: 466.27 $(M+H)^+$.

Conclusion

The yields of various phenyl thiazolyl-triazine derivatives were improved using microwave heating under solvent-free conditions. Besides, the technique has the advantage of being simple and allows the synthesis of this hybrid class of compounds in a minimum reaction time since the reactions were carried out under pressure in closed vessels, it minimizes the possibilities of material loss and so the occurrence of impurities too.

The work described in this paper also concludes that thiazoles besides being good antibacterial, they can also serve as active antimalarial. Among the synthesized hybrid thiazolyl-triazines, seven compounds have shown good antimalarial activity. From this study it can be suggested that incorporation of amines in the same hybrid structure, which can exhibit all possible kinds of interaction with amino acid residues Ile 14, Asp 54, Ile 164, and Arg122 might help in developing new antimalarial drugs with elevated activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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